SIMPLE PREPARATIONS OF C-4-*TERT*-BUTYLATED NADH/NAD$^+$ ANALOGS

Agnès Anne
Laboratoire d'Electrochimie Moléculaire, Université de Paris 7, URA 438, 2, Place Jussieu, 75251, Paris Cedex 05, France

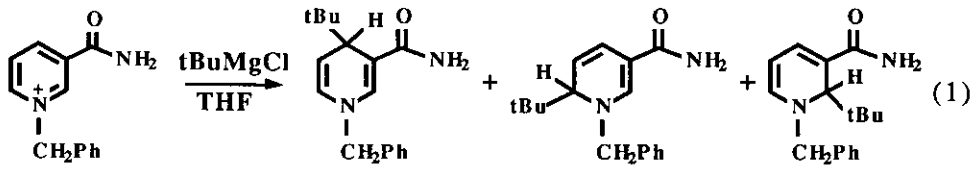
Abstract- The addition of *tert*-butyl Grignard reagent to the 1-benzyl-3-carbamoylpyridinium salt (**1**) gives a mixture of the C-4-(**2**), C-6-(**3**), and C-2-*tert*-butylated dihydronicotinamides (**4**) in which the desired 1,4-isomer predominates. Stable cristalline 1-benzyl-4-*tert*-butyl-1,4-dihydronicotinamide (**2**) can be easily isolated. Oxidation of the product with the 1-benzyl-3-cyanoquinolinium ion (**6**) was found to be strongly solvent-dependent. In acetonitrile, exclusive hydride transfer gives the corresponding C-4-*tert*-butylated pyridinium ion (**5**). In methanol, an interesting *tert*-butyl transfer from 1,4-dihydronicotinamide (**2**) to quinolinium (**6**) occurs competitively, and predominates in the presence of a catalytic amount of formic acid; the resulting C-4-*tert*-butylated 1,4-dihydroquinoline derivative (**8**) can be readily isolated.

The C-4 alkylated 1,4-dihydropyridines have attracted much recent interest both for their use as model compounds of the dihydronicotinamide coenzyme NADH,[1-3] and for their synthetic and pharmacological significance.[1,4] In contrast with the well documented 4-substituted Hantzsch-ester 1,4-dihydropyridines which are easily prepared,[1] synthetic routes to the new important classes of *N*-substituted 4-alkyl(4-R)-1,4-dihydronicotinamides, have only been reported in a few cases, R being to the author's best knowledge the methyl group [2,3,5] or bulkier hydroxyalkyl groups.[6] Whereas the performance of the 4-methyl-1,4-dihydronicotinamides as NADH mimics has been evaluated and compared with those of existing model systems, there has been no report of reactions involving C-4 encumbered 1,4-dihydronicotinamides as formal hydride donors.

Since it is of interest to know if the introduction of a bulky substituent to the 4 position of the models of NADH/ NAD$^+$ redox couple may induce changes in their reactivities, the novel structurally simple sterically hindered system 1-benzyl-4-*tert*-butyl-1,4-dihydronicotinamide /1-

benzyl-4-*tert*-butylnicotinamide ion has been developed. The preparation of the oxidized form represents the first example of a pyridinium (or analogue) bearing both an electron withdrawing substituent at the 3-position and a bulky alkyl group at the 4-position. Examination of the reaction of the reduced form with the 1-benzyl-3-cyanoquinolinium NAD^+ analogue reveals that this new compound may act as a hydride donor and /or a *tert*-butylating reagent depending upon the nature of reaction solvent.

Addition studies of Grignard reagents to some 1-alkylpyridinium salts reported earlier,[7] show that this method should provide a convenient route to the desired isomeric compound (**2**) (eq.1). The readily available 1-benzyl-3-carbamoylpyridinium chloride (**1**) was treated in tetrahydrofuran with *tert*-butylmagnesium chloride under usual conditions.



1 **2** **3** **4**

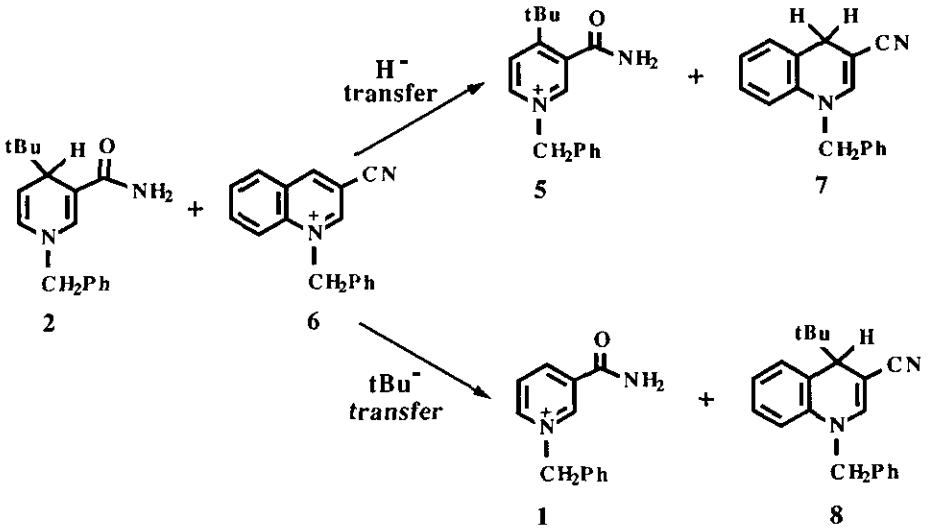
Moderate reactivity was observed, and some of the starting pyridinium was recovered after work-up even when longer reaction times were used. Nevertheless, the reaction was found to be regioselective, affording the 1,4-dihydronicotinamide (**2**) as the predominant product, and giving the two other isomers, 1,6-dihydronicotinamide (**3**) and 1,2-dihydronicotinamide (**4**) in relative ratios of 75/10/15 respectively as shown by nmr analysis of the reaction mixture after work-up, with no evidence of equilibration between isomers. That the ring alkylation is directed to the 4 position might result in part from the steric hindrance of the extremely bulky *tert*-butyl group to approach the 2 and 6 positions due to the proximity of the benzyl group; however other factors must govern the regioselectivity since the percentage of the 1,2-isomer was always higher than that of the 1,6-isomer. In the case at hand, only the 4-addition was considered important, and despite the moderate yield obtained for (**2**) after purification (ranging from 30 to 35%), its isolation and purification were readily achieved. In contrast, products (**3**) and (**4**) could not be obtained in analytical purity, and substantial losses of materials were caused by repeated preparative chromatography. The 1,6-isomer (**3**) could not be made free from contamination of a very small amount of (**2**), while decompositions occurred during isolation-purification procedures in the case of the 1,2-isomer (**4**). However, these two compounds were found to be sufficiently pure for spectroscopic purposes.

The structures of (**2**), (**3**) and (**4**) were easily assigned from their spectral proprieties. In ^{1}H-nmr, the *N*-methylene protons of either (**3**) or (**4**) show an AB double doublet as a result of diastereotopic splitting, whereas those in (**2**) appear as a singlet. For (**2**), one proton at C-4 is evidently missing and the coupling pattern is in accord with the assigned structure. Differentiation between the 1,2- and 1,6-isomers is in particular provided by the signal from the

proton attached to the sp^2 carbon adjacent to the nitrogen, which appears as a doublet in (**4**) (δ 6.85) with a strong coupling (J= 6.2 Hz) while it gives rise to a singlet in (**3**) (δ 7.44). The uv-visible spectroscopic data of (**2**), (**3**) and (**4**) were those respectively expected for 1,4-, 1,6-, and 1,2-dihydropyridine derivatives.[8]

Oxidation of 1-benzyl-4-*tert*-butyl-1,4-dihydronicotinamide (**2**) utilizing 1-benzyl-3-cyanoquinolinium (**6**), which is known as a relatively strong dehydrogenating reagent among various NAD^+ analogues,[9, 10] proved to be an efficient and practical route to the corresponding pyridinium form, 1-benzyl-4-*tert*-butylnicotinamide ion (**5**), when acetonitrile was selected as the reaction solvent (Scheme I). Treatment of 1,4-dihydronicotinamide (**2**) (0.3 mmol) with 1 equivalent of 1-benzyl-3-cyanoquinolinium (**6**) (tetrafluoroborate salt) gave at room temperature in the dark the desired pyridinium (**5**), together with 1-benzyl-3-cyano-1,4-dihydroquinoline (**7**), in a quantitative yield as determined by hplc analysis. The reaction proceeded somewhat slower (16 h) than the reaction of C-4-unsubstituted 1-benzyl-1,4-dihydronicotinamide with (**6**), investigated earlier under the same conditions,[10] probably due to steric hindrance to the hydride transfer. The pyridinium salt (**5**) was readily isolated in good yield; its structure was confirmed by elemental and ^{1}H-nmr analyses, thus providing unequivocal structural proof for (**2**).

Scheme I.



During the investigation of reaction of (**2**) with (**6**), a striking solvent effect was observed based on the hplc analysis of the product distribution in the resulting reaction mixture. When passing from acetonitrile to methanol, unexpected dealkylation of (**2**) occurred in competition with the formal hydride transfer (Scheme I), as shown by the formation of dealkylated pyridinium (**1**) and pyridinium (**5**), in a ratio of 5/4. This new reaction pathway appeared to be equivalent to a formal *tert*-butyl anion migration from (**2**) to (**6**), and was used to prepare 1-benzyl-4-*tert*-butyl-3-

cyano-1,4-dihydroquinoline (**8**), which is the first reported representative of 1,4-dihydroquinolines bearing a bulky alkyl group at the 4 position. For a convenient isolation of (**8**), reaction conditions were slightly modified to favor predominantly the dealkylative pathway, and quinolinium (**6**) was used as its readily available bromide form, which presents a high solubility in protic solvents. Compound (**2**) was treated with (**6**) in methanol containing a catalytic amount (5 mol %) of formic acid ; complete oxidation was achieved within a short reaction time (3 h), and afforded quantitatively a mixture of the pyridiniums (**1**) and (**5**), in a ratio of 7/3. In addition, the reaction yielded dihydroquinoline (**7**) along with a pale yellow precipitate, which was easily identified as the C-4-*tert*-butylated dihydroquinoline (**8**) (50% isolated yield) by mass and ^{1}H-nmr spectroscopies as well as elemental analysis.

EXPERIMENTAL SECTION

The reaction involving the Grignard reagent was conducted in oven-dried glassware under an atmosphere of dry nitrogen.

^{1}H-Nmr spectra were run on a Brucker AC250 spectrometer. Uv-visible spectra were recorded on a Hewlett-Packard 8452 diode array spectrophotometer. High performance liquid chromatography (hplc) was performed with a Gilson gradient system equipped with a Waters Novapak C18 analytical column (3.9 mm i.d. x 15 cm, 4 µm particule size) monitoring the eluate at 260 or 330 nm. The mobile phases were (A) 70% MeOH in water, and (B) 70% MeOH in water containing 0.1 M NaCl; the gradient consisted of a 15-min linear step from 0% to 100% B, followed by a 5-min linear step to initial conditions (100% A). Flow-rate was 1.2 ml min^{-1}. The relative amounts of products resulting from reaction of 1-benzyl-4-*tert*-butyl-3-carbamoyl-1,4-dihydropyridine (**2**) with 1-benzyl-3-cyanoquinolinium (**6**) were determined by means of the corresponding hplc peak areas corrected for variations in the extinction coefficients among the compounds.

1-Benzyl-3-carbamoylpyridinium chloride (**1**), 1-benzyl-3-cyanoquinolinium bromide (**6**) and tetrafluoroborate, and 1-benzyl-3-cyano-1,4-dihydroquinoline (**7**) were prepared according to previously described procedures.[10]

Reaction of 1-Benzyl-3-carbamoylpyridinium 1 with *tert*-Butylmagnesium Chloride in THF.

To a stirred suspension of 1-benzyl-3-carbamoylpyridinium chloride (2.48 g, 10 mmol) in 20 ml of dry THF, a solution of *tert*-butylmagnesium chloride (1.29 g, 11 mmol) in 5.2 ml of ether was added through a syringe over 10 min with cooling in an ice bath. The mixture was stirred for 1 h at room temperature and hydrolyzed with an aqueous 30% NaOH solution (5 ml). After addition of ether (50 ml), the yellow reaction mixture was filtered and washed with

dichloromethane (40 ml). The filtrate and the dichloromethane washing were combined, and concentrated *in vacuo* on a rotary evaporator to remove organic solvents. The residual liquid was partitioned between dichloromethane (50 ml) and water (50 ml). The dichloromethane layer was washed twice with water to extract recovered starting material (**1**), dried over magnesium sulfate, and concentrated *in vacuo* to leave 1.2 g of a yellow solid which was shown by ^{1}H-nmr spectroscopy to contain the 1,4-dihydropyridine (**2**), 1,6-dihydropyridine (**3**) and 1,2-dihydropyridine (**4**) in the ratio *ca.* 75/10/15. Such ratios were measured by means of the signals due to the *tert*-butyl protons for (**2**) and to the H-4 proton for the two other isomers. The solid was taken up in *ca.* 20 ml of MeOH at 30-40°C; the solution was filtered and water was added until cristals began to appear. The flask was placed in a refrigerator for a while and the precipitate of the 1,4 isomer (**2**) which formed was collected and washed with water. Recristallization from 40% aqueous methanol afforded 0.9 g (33% yield) of 1-benzyl-4-*tert*-butyl-3-carbamoyl-1,4-dihydropyridine (**2**) as pale yellow pallets. To obtain the 1,2- and 1,6-isomers, the above filtrate was concentrated *in vacuo* on a rotary evaporator. Subsequent preparative layer chromatography (silica gel, 5% MeOH-ethyl acetate) of the residue afforded a yellow band (Rf 0.75) and a fluorescent band (Rf 0.59) corresponding to the 1,2- and 1,6-isomers respectively, contaminated with the 1,4-isomer (Rf 0.65). Repeated layer chromatography gave (**4**) as a yellow solid and (**3**) as a white solid in small amounts. Further treatment for purification resulted in consumptions of the compounds, but those were essentially pure from thin layer chromatography and spectroscopic analyses.

1-Benzyl-4-*tert*-butyl-3-carbamoyl-1,4-dihydropyridine 2.

mp 146°C; ^{1}H-nmr ($CDCl_3$) δ 7.31 (1H, s, H-2), 7.30-7.15 (5H, m, C_6H_5), 6.02 (1H, dd, $J_{6,5}$= 7.6 Hz, $J_{6,4}$= 1.3 Hz, H-6), 5.51 (2H, bs, NH_2), 4.81 (1H, dd, $J_{5,6}$= 7.6 Hz, $J_{5,4}$= 5.9 Hz, H-5), 4.36 (2H, s, NCH_2), 2.99 (1H, dd, $J_{4,5}$= 5.9 Hz, $J_{4,6}$= 1.3 Hz, H-4), 0.78 (9H, s, $C(CH_3)_3$); uv-vis, λ_{max} (nm) (ε_{max} (M^{-1} cm^{-1})), MeCN, 322 (5400); CI-ms (NH_3) m/z (relative abundance) 271 (MH^+, 100), 213 (M-$C(CH_3)_3$, 18). *Anal.* Calcd for $C_{17}H_{22}N_2O$: C, 75,52; H, 8.20; N, 10.36. Found: C, 75,62; H, 8.23; N, 10.29.

1-Benzyl-6-*tert*-butyl-3-carbamoyl-1,6-dihydropyridine 3.

^{1}H-Nmr ($CDCl_3$) δ 7.44 (1H, s, H-2), 7.30-7.15 (5H, m, C_6H_5), 6.36 (1H, dd, $J_{4,5}$= 9.6 Hz, $J_{4,6}$= 1.1 Hz, H-4), 5.35 (2H, bs, NH_2), 4.97 (1H, dd, $J_{5,4}$= 9.6 Hz, $J_{5,6}$= 5.8 Hz, H-5), 4.50 (2H, app q, AB system, J= 15.8 Hz, NCH_2), 3.68 (1H, dd, $J_{6,5}$= 5.8 Hz, $J_{6,4}$= 1.1 Hz, H-6), 0.92 (9H, s, $C(CH_3)_3$); uv-vis, λ_{max} (nm) (ε_{max} (M^{-1} cm^{-1})), MeCN, 340 (4900), 262 (7700); CI-ms (NH_3) m/z (relative abundance) 271 (MH^+, 100), 213 (M-$C(CH_3)_3$, 35).

1-Benzyl-2-*tert*-butyl-3-carbamoyl-1,2-dihydropyridine 4.

^{1}H-Nmr ($CDCl_3$) δ 7.35-7.10 (5H, m, C_6H_5), 6.85 (1H, d, J= 6.2 Hz, H-6), 6.45 (1H, dd, J= 6.2 Hz, J= 1.1 Hz, H-4), 5.31 (2H, bs, NH_2), 4.95 (1H, app t, J= 6.4 Hz, H-5), 4.50 (2H, app q, AB system, J= 16 Hz, NCH_2), 4.50 (1H, 2xd, J=0.45 Hz, H-2), 0.91 (9H, s,

$C(CH_3)_3$); uv-vis, λ_{max} (nm) (ε_{max} (M^{-1} cm^{-1})), MeCN, 374 (3900); CI-ms (NH_3) m/z (relative abundance) 271 (MH^+, 100).

1-Benzyl-4-*tert*-butyl-3-carbamoylpyridinium Tetrafluoroborate 5.

In a 10 ml flask equipped with a serum cap, 1-benzyl-3-cyanoquinolinium tetrafluoroborate (99.6 mg, 0.3 mmol) was placed with 3 ml of MeCN and nitrogen gas was bubbled through the solution. Then 1-benzyl-4-*tert*-butyl-3-carbamoyl-1,4-dihydropyridine (**2**) (81 mg, 0.3 mmol) was added to the solution with stirring and the flask was sealed with a serum cap immediately. Upon mixing, an intense red colour developed instantaneously and then faded, suggesting the formation of a charge transfer complex between the two reactants.[11] The mixture was allowed to react for 16 h at room temperature in the dark under a stream of nitrogen. Hplc analysis of the crude reaction mixture indicated that the 1,4-dihydroquinoline (**7**) and the pyridinium (**5**) were formed in quantitative yields. The solvent was evaporated and the residue was washed with several portions of ether. Then the residue was taken up in dichloromethane-water. The aqueous layer was separated and the organic layer was extracted two times with water. The combined aqueous layers were evaporated on a rotary evaporator leaving a partially cristallized material. Scratching and trituration with ether until solid, followed by copious washing with ether, afforded 1-benzyl-4-*tert*-butyl-3-carbamoylpyridinium tetrafluoroborate (**5**) (74 mg, 70%) which was shown to be essentially pure from spectral and hplc analyses. Recristallization from ethanol-ether gave an analytical sample of (**5**) as white needles: mp 174°C; ^{1}H-nmr (DMSO-d_6) δ 9.24 (1H, d, J= 1.3 Hz, H-2), 9.07 (1H, dd, $J_{6,5}$= 6.7 Hz, $J_{4,6}$= 1.1 Hz, H-4), 8.39 (2H, bs, NH_2), 8.22 (1H, d, $J_{5,6}$= 6.7 Hz, H-5), 7.6-7.4 (5H, m, C_6H_5), 5.79 (2H, s, NCH_2), 1.46 (9H, s, $C(CH_3)_3$); uv-vis, λ_{max} (nm) (ε_{max} (M^{-1} cm^{-1})), MeCN, 262 (6400). *Anal.* Calcd for $C_{17}H_{21}N_2OBF_4$: C, 57.33; H, 5.94; N, 7.87. Found: C, 57.03; H, 5.90; N, 7.80. Hplc t_r = 8.0 min.

1-Benzyl-4-*tert*-butyl-3-cyano-1,4-dihydroquinoline 8.

1-Benzyl-3-cyanoquinolinium bromide (**6**) (97.5 mg, 0.3 mmol) was allowed to react with compound (**2**) (81 mg, 0.3 mmol) in 3 ml of MeOH containing formic acid (5 mol %) for 3 h according to the above procedure. The intense dark colour which appeared instantaneously on mixing turned bright yellow. After 1 h stirring, a pale yellow precipitate formed. Over this period of time, samples were removed periodically, diluted suitably, and were subjected to hplc analysis: the decrease of the peaks due to the reactants was essentially accompanied by the increase of four peaks, corresponding to the non-alkylated and C-4-alkylated pyridiniums (**1**) and (**5**), the 1,4-dihydroquinoline (**7**) (as checked with authentical samples), and the new C-4-alkylated-1,4-dihydroquinoline (**8**); these control runs showed that (**1**) and (**8**) were formed predominantly in comparable amounts, an approximately constant ratio of 70/30 being obtained for either (**1**) and (**5**), or (**8**) and (**7**). After completion of the reaction, the precipitate was collected by filtration, washed twice with water and then with cold methanol to give (**8**) as

a white fluffy powder (45 mg, 50%): mp 138°C; ^{1}H-nmr ($CDCl_3$) δ 7.35 (1H, s, H-2), 7.4-7.0 (9H, m, H-5, H-6, H-7 and C_6H_5), 6.87 (1H, d, $J_{8,7}$= 8 Hz, H-8), 4.83 (2H, app q, AB system, J= 16.2 Hz, NCH_2), 3.38 (1H, s, H-4), 0.9 (9H, s, $C(CH_3)_3$); uv-vis, λ_{max} (nm) (ε_{max} (M^{-1} cm^{-1})), MeCN, 316 (10250), 232 (8750); CI-ms (NH_3) m/z (relative abundance) 303 (MH^+, 100), 245 (M-$C(CH_3)_3$, 10). *Anal.* Calcd for $C_{21}H_{22}N_2$: C, 83.40; H, 7.33; N, 9.26. Found: C, 83.51; H, 7.29; N, 9.21. Hplc tr = 20.45 min.

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